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(Article begins on next page)

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LIFE CYCLE ASSESSMENT APPLIED TO POLYHYDROXYALKANOATES PRODUCTION PHASES: A MINI REVIEW

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*Abstract:*Polyhydroxyalkanoates (PHAs) are bio-based polyesters that are natural, renewables, biocompatible and biodegradable while having similar properties to commonly used plastic. However, their industrial production is still more expensive than the petroleum-based one. To make PHAs marketable on a large scale, it is therefore necessary to optimize every single phase of their production. This process mustbe accompanied by environmental assessments, to verify that PHAs are a greener alternative to conventional plastic. To do this, the best tool currently available is Life Cycle Assessment (LCA). The purpose of this study is to examine the state of the art regarding the application of the LCA methodology to all phases of PHAs production.

1. Introduction

Polyhydroxyalkanoates (PHAs) are polyesters naturally produced by numerous microorganisms through a fermentation process of sugar or lipids. They are accumulated as granules in cell cytoplasm and serve both as source of energy and carbon storage for the microorganism. These polymers have gained more and more attention in the last decade, due to their physical and mechanical properties, which resemble those of conventional plastics, such as polyethylene and polypropylene (Ciesielski et al., 2015). Therefore, PHAs are seen as a greener and more sustainable alternative to fossil fuel plastic derivatives, because they are natural, renewable, biocompatible, and completely biodegradable. However, their large-scale production and market presence is still precluded because of their high production cost, which is estimated to be about 5 times the cost of their petrochemical counterparts (Cristóbal et al, 2016). Rodriguez-Perez et al., (2018) indicated feedstock as the main hindrance of affordable PHAs production. Therefore, its choice is of primary importance to obtain marketable PHA. However, both upstream and downstream costs add up determining the PHAs high production cost (Pagliano et al., 2021). So, every manufacturing phase needs to be made efficient. In this context, BioLaMer aim to answer these necessities. This is a pioneering project, whose target is to produce PHA in a cost-effective and environmentally sustainable way, by improving the bioreactor process efficiency. Therefore, two important aspects that define the success of the BioLaMer technologies are the environmental performance and sustainability. University of Bologna is leading one of the work packages of the project by applying its expertise in Life Cycle Thinking (LCT) and Analysis (LCA) to assess the environmental sustainability of BioLaMer system, which allow to understand and take into consideration the environmental impacts of the process, and to guide its

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development. This review paper, which is an update of a previous works (Vogli et al., 2020), aims to present the results of a detailed literature research on LCA analysis applied to the various phases of PHA production. These phases can be grouped into three main steps: the feedstock selection and processing, the biorefinery PHA synthesis and the downstream process of PHA extraction and purification.

2. LCA of feedstock

Polyhydroxyalkanoates are produced by bacteria when they are provided with controlled diet having an excess of carbon and a limited supply (starvation) of phosphate and/or nitrogen sources (Anderson & Dawes, 1990). Different sources of carbon have been used as PHA biorefinery feedstock, from agricultural crops to waste streams. Figure 1 represent the carbon source considered by 34 studies on LCA of PHA production (some of which considered more than one carbon source).

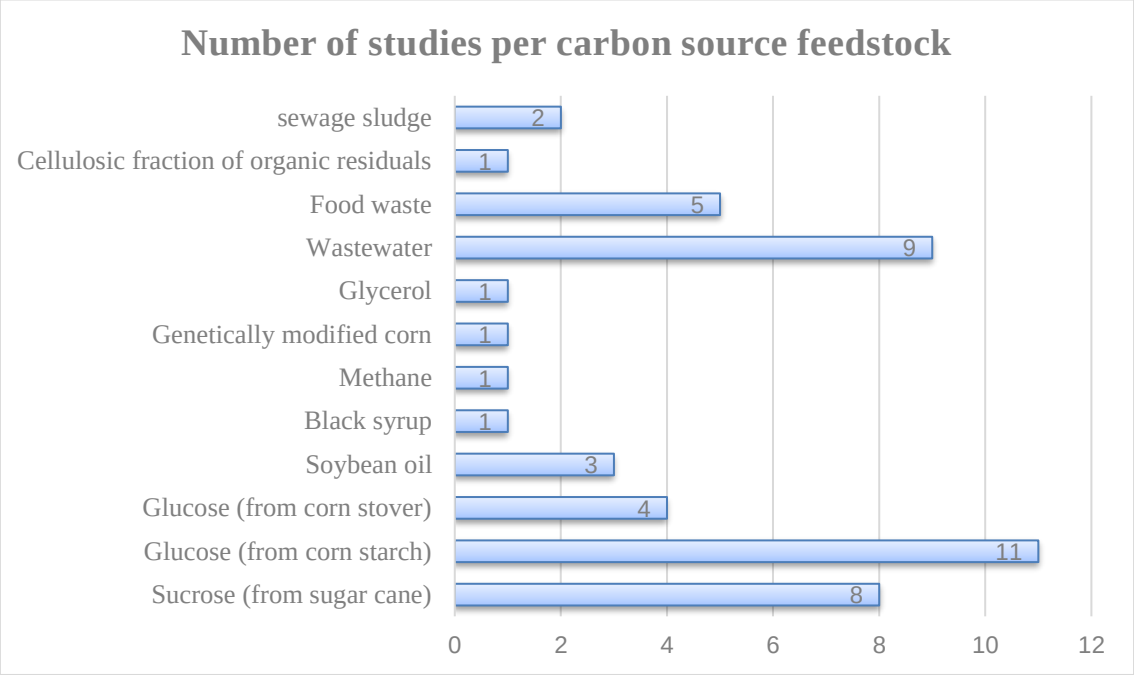

Figure 1: number of studies per carbon source feedstock

Feedstock is an important part of PHA production process. In fact, its choice and treatment (for example its co-use for energy production) can greatly vary PHA production environmental impact, and even make the material a net carbon sink (Dietrich et al., 2017; Jiang et al. 2016).

Biopolymers produced at industrial scale typically use purpose grown feedstocks, such as corn (Kendall, 2012), but it has been shown that the feedstock growth phase itself can have the highest impact on several environmental categories. An example reported by Cristobal (Cristóbal et al., 2016), stated that sugar production from sugarcane and corn starch accounted for more than two third of the value in aquatic marine eutrophication, terrestrial eutrophication, human toxicity, and particulate matter. A few attempts have also been made using genetically modified feedstock (Kurdikar et al., 2008). Therefore, alternative carbon sources are sought. The improvement on biorefinery's technology and the development of mixed microbial culture PHA production, made it possible to also take into consideration raw materials other than agricultural ones, such as food waste or wastewater. Using waste streams as feedstock has the additional benefit to simultaneously address waste treatment and disposal problem (De Donno Novelli et al., 2021). Over the years, the number of studies that considered waste streams as carbon sources increased,

supporting preservation of biodiversity initiatives, while the use of agriculture crops to produce biopolymers is reduced (Samorì et al., 2019; Valentino et al., 2019; Vega et al., 2019; Yadav et al., 2020)

3. LCA of PHA production

Great heterogeneity can be found in current literature regarding the application of LCA to PHA production both in its scope (the focus could be on production optimization or on the comparison with their petrochemical counterpart) and in methodological choices. On this matter, a 2016 review by Cristobal and colleagues (Cristóbal et al., 2016) report that most of the studies limited the impact categories, while product environmental footprint (PEF) establishes fourteen of them, including human toxicity. Moreover, most of the reviewed studies' results considered data from PHA production in laboratory or pilot facilities. They also indicate that the two most common chosen impact category are climate change and acidification. The first one is evaluated assessing the kg CO_2 eq/kg PHA and showed great variability in the results, depending on the feedstock choice and the end-of-life treatment. The lowest values, that ranged from -2.3 to 2.3 kg CO_2 eq/kg PHA, are obtained when the burn of waste streams lead to energy recovery. On the other hand, high end climate change emissions are reported when the feedstock of choice show a lower productivity when compared to sugar, as with rapeseed oil, whose kg CO_2 eq/kg PHA ranged from 5 to 6.9.

Lopez Arena and colleagues (Lopez-Arenas et al, 2017) focus their work on fermentation reaction dynamics, applying models to determine its best operation mode and feeding strategy. The importance of this stage has also been underlined by Hermann (Hermann et al., 2007) who claim that the environmental impacts of the studied processes depend to a large extent on the productivities, yields, and concentrations assumed for the fermentation stage. Moreover, this phase appears relevant also considering that Chen and Patel (Chen & Patel, 2012) claim the energy intensive needs for this process, that they state is the highest contributor to climate change potential. Of the different operating conditions analysed by Lopez Arena (Lopez-Arenas et al, 2017), the fed-batch case showed the best results both from a cost – benefit profile and an environmental perspective. The fed-batch option, in fact, displays similar CO_2 emissions (1.7 PHB vs 1.9 kg CO_2/ kg) in comparison to the batch fermentation model, while exhibiting lower energy requirement. The energy requirement, in fact, is reported to be 10 MJ/kg PHB for the fed-batch option and 29.2 MJ/ kg PHB for the batch operation mode. Moreover, the fed-batch option exhibited the lowest water consumption of the of the analysed fermentation options. This last point is interesting considering that the process' high water demand has been indicated as a potential concern for biobased chemicals, for the impact that it can have on stored fresh water (Pawelzik et al, 2013; Harding et al, 2007). Therefore, the development of a bioreactor and a fermentation process that saves water is of primary importance. This can lead to even better results if combined with the right feedstock. Cristóbal (Cristóbal et al., 2016) reported a lower water resource depletion for sugarcane (2.35 m3 water-eq) than for corn starch (3.91 m3 water-eq).

Another aspect that varies both the environmental impacts and the cost-benefits of the process is the microbial culture, that can be pure or mixed. Also, recombinant strains have been used, aiming to increase fermentation yield. Several PHA accumulating microorganism has been utilized in pure cultures for biopolymer production. Among them, *Escherichia coli* (Xuan Jiang et al., 2006; Martin et al., 2014; Zhou et al., 2012); *Methylocystis hirsute* (López et al. 2018) *Ralstonia eutropha* (Akiyama et al., 2003). However, the advantage of PHA from pure-culture in comparison to fossil-based polymers are hindered by the expensive and energy demanding feedstock, e.g. glucose or acetic acid, used during the fermentation process (Patel et al., 2005). Moreover, they require sterile cultivation condition, which also increases the production cost (Gholami et al., 2016). Therefore, if PHA production by pure microbial cultures has been initially

developed and assessed in several LCAs during the previous decade, nowadays the technology is focusing more on mixed-culture for PHAs production (Heimersson et al., 2014). Gurieff (Gurieff & Lant, 2007) compared mixed culture PHA production with pure culture PHA production and HDPE. For both pure and mixed-culture PHA production most of the non-renewable CO_2 -eq emissions derived from the energy usage. While the energy expenditure aspect seems to be comparable between pure and mixed culture PHA production, an important aspect that needs to be taken into consideration is that with the latter it is possible to use waste streams as fermentation substrate, and the sterile fermentation conditions required by pure-culture are not needed. Waste streams often used as feedstock for PHA production are wastewater and food waste (Battista et al, 2020; Nielsen et al, 2017; Valentino et al, 2019). So, mixed cutures seems to have a large potential in optimizing PHAs production problems. On this matter, Heimersson et al. (2014) gave some recommendation for LCAs practitioners while analyzing a mixed culture. Among them, the authors reported to handle multifunctionality throught partial subdivision coupled with substitution or allocation and advise to include eutrophication and land use impact categories when studied system includes a comparison to a grain-based polymer.

Methodological choices can also affect impact results. Among them, one important aspect in relation to biopolymers is the assessment of biogenic carbon storage in the polymer itself. In the literature, low grade of climate change impact values (0.49 kg CO_2 eq/kg PHA) are reported from studies were the temporary storage of atmospheric CO_2 in the polymer is accounted as carbon sequestration (Cristóbal et al., 2016). However, the concurrent existence of different approaches of evaluation can lead to non-comparable results. This particularly applies regarding how to account for the period of the carbon storage. This releasing delay is in fact the one that postpone the emissions' radiative force (Hoxha et al 2020). An example of the different existing approaches can be found confronting the ISO 14067 (ISO 14067:2018) and the ILCD Handbook (2010). The first one account for the biogenic carbon storage time only when it occurs for at least 10 years and requires reporting it as a separate calculation. On the other hand, the ILCD Handbook account for delayed emissions within a 100-year period by applying a linear discounting for climate change emissions of 1%/year. Moreover, Pawelzik and colleagues emphasized that the account for bio-based carbon storage is particularly controversial when "cradle-to-factory gate" system boundaries are considered, because it excludes the end-of-life disposal of the product, and therefore the carbon's fate (Pawelzik et al, 2013). Furthermore, other challenges are reported in the literature regarding LCA analysis of PHAs production using waste feedstock. Among them, the selection of the allocation method when multifunctionality occurs, the considerable dependence of the result on geographical specificity (in particular on the sensitivity to the selected energy mix), and the lack of inventory data for new technologies (Heimersson et al. 2014).

4. LCA of the downstream process

PHAs downstream processing usually comprehend a physical separation of the biomass which precedes the actual extraction step, and sometimes a pre-treatment step between them (Kosseva & Rusbandi, 2018). During these steps, various substances or hazardous solvents may be used, making the downstream process a bottleneck in the PHA value chain, due to the high indirect energy consumption and high process energy derived by these chemicals (Álvarez-Chávez et al., 2012; Chen and Patel, 2012). The high potential of optimization of downstream processing has also been affirmed by Gurieff, who stated that both financial and environmental costs of PHA mixed culture process was related to energy used for the downstream processing (Gurieff & Lant, 2007). López-Abelairas compared the environmental performance of recovery treatments, based on acid or alkaline treatments. The alkaline sodium hydroxide and the acid sulfuric acid treatment had the lowest GHG emissions (respectively 4.08 kg CO_2 eq/kg PHB and 6.27 kg CO_2 eq/kg PHB) and operational costs. Between them the sulfuric acid treatment showed high recovery efficiency, high polymer purity and low polymer degradation. Therefore, acid

treatment was proposed by the authors as a viable downstream processing alternative to the classic chloroform extraction (López-Abelairas et al., 2015). Moreover, also Fernández-Dacosta evaluated three treatments for PHA release, based on alkali, surfactant- hypochlorite and solvent treatments. Among them the less favourable one is the solvent extraction both for the high energy requirements (156 MJ/kg PHB) and GHG emissions (4.30 kg CO_2-eq/kg PHB). On the other hand, the alkali treatment showed a global warming potential of 2.4 kg CO_2-eq/kg PHB and non-renewable energy use of 106 MJ/kg PHB, making it the most convenient one amidst the three evaluated downstream processing routes (Fernández-Dacosta et al., 2015).

An interesting insight about PHA downstream processing was provided by Saavedra del Oso and colleagues. They analysed eight PHA downstream alternatives' hotspot, both from an environmental and a tecno-economic point of view. From this investigation emerged that the most promising technology in terms of environmental performance appears to be mechanical disruption, even when the electricity mix is carbon intensive. For low- grade of PHA purification, surfactant treatment appears to be the most promising method. In fact, surfactants and sodium hydroxide are a good environmental alternative compared to organic solvent extractions. Nevertheless, the crystallization required for surfactant recovery can significantly reduce the environmental performance of the process. Higher performances can be obtained through chemical digestion or solvent extraction. The first has to be optimized by adding a chemical recovery unit. The large amounts of energy for solvent recovery required for the second are justified when low impurity in the products is required (Saavedra del Oso et al., 2021).

5. Conclusions

With characteristics that resemble those of conventional plastic, PHAs are seen as a promising bio-based alternative. However, their large-scale market is hindered by their high production cost. Therefore, to make PHA commercially suitable, every phase of its production needs to be cost-effective. So, advancement in the efficiency of the process is a focal point of new research projects, as BioLaMer. Over the years, LCA analysis has accompanied the production process' development to evaluate PHA sustainability compared to petroleum- based plastic. In fact, life cycle thinking helps verifying that the bio-based polymer remains a greener alternative. On this matter, this study aims to examine and summarize the current literature regarding the application of LCA analysis on PHA production.

Concerning feedstock, research showed that the focus has shifted from agricultural crops to waste. This not only makes the process circular, but simultaneously address the problem of waste treatment and disposal. With reference to both the production phase and the downstream processes, LCA studies helps choosing between possible alternatives and assist locate critical aspects on which attention must be placed during further development. For the production phase appear of particular importance the water consumption and the energy source during the fermentation process. Instead, extracting methods in downstream processes vary according to the needed grade of PHA purification, which depends on the final product application.

Another aspect emerged from this research is that there is still great methodology heterogeneity. One of the most interesting and crucial one while analysing biopolymers is the account for carbon storage in the product, which is faced with different approaches. Still, LCA confirm itself as a key tool for an efficient optimisation of the production process in the technology development phase.

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